REMARKS

The specification has been amended to change trade names Triton X-100, Triton X-114, and Igepal CA-630, to their generic names octylphenol ethylene oxide condensate, octylphenoxypoly (ethyleneoxy) ethanol, and tert-octylphenoxy poly(oxyethylene)ethanol, respectively. The generic names for these detergents are provided by the Sigma Product Information descriptions (available as PDF documents via the World Wide Web at <www.sigmaaldrich.com> as accessed July 1, 2004) attached hereto as Exhibit A. The date following the list of References in each Product Information document refers to the date on which each document was approved for posting to the Sigma-Aldrich website (information from Sigma-Aldrich technical services); the dates of approval for the Product Information sheets for each detergent predates the filing date of the instant application, indicating that this information was publicly available prior to the filing date of the application. The chemical compositions of the trademarked detergents are inherent properties of the detergents and thus are not new matter.

Claims 1-20 were pending in this application. Claims 2, 4-8, and 12-17 have been canceled without prejudice. Applicants reserve the right to prosecute the subject matter of any canceled claim. Claims 1, 3, and 11 have been amended to recite the presence of a chaotropic agent. Support for amended claims 1, 3, and 11 can be found in the specification at, e.g., page 30, lines 15-16. Claims 9 and 10 have been amended to change trade names Triton X-100, Triton X-114, Igepal CA-630, and ROTOLYTES to their generic names octylphenol ethylene oxide condensate, octylphenoxypoly (ethyleneoxy) ethanol, (octylphenoxy) polyethoxyethanol, and ampholytes, respectively. The chemical compositions of the trademarked detergents are inherent properties of the detergents and thus are not new matter (see Sigma Product Information descriptions attached as Exhibit A).

New claims 21-38 have been added to clarify what Applicants regard as the invention. Support for new claims 21-38 can be found in the specification at, e.g., page 4, lines 18-24,

page 6, lines 4-8, and page 30, lines 15-16. Upon entry of this Amendment, claims 1, 3, 10-11, and 18-38 will be pending.

Entry of the foregoing amendments and consideration of these remarks are respectfully requested.

1. THE OBJECTIONS TO THE DISCLOSURE OVER USE OF TRADEMARKS SHOULD BE WITHDRAWN

The disclosure is objected to for reciting trademarks for detergents such as TRITON and IGEPAL. Applicants have amended the specification to specify the chemical name of the trademarked products objected to. The chemical compositions of the trademarked detergents are inherent properties of the detergents and are not new matter (see Sigma Product Information descriptions attached as Exhibit A).

Accordingly, Applicants respectfully request that the objection to the disclosure for recitation of the trademarks of detergents TRITON and IGEPAL should be withdrawn.

2. THE CLAIM REJECTIONS UNDER 35 U.S.C. § 112, 2nd PARAGRAPH, SHOULD BE WITHDRAWN

The Examiner rejects claims 1 and 10 as being indefinite for the recitation of "collecting one or more fractions". The Examiner contends that it is not clear whether the "one or more fractions" are pooled or kept separate. The Examiner states that separate fractions could correspond to pure single chaperone proteins, with a mixture of chaperone proteins arising from the pooling of fractions. Applicants respectfully disagree and assert that the specification and claims clearly distinguish between "collecting" and "pooling" fractions after free solution isoelectric focusing (FS-IEF).

As described in the specification, collecting fractions is a step distinguishable from pooling fractions. For example, the specification at page 15, lines 25-35 describes the collection of fractions containing chaperone proteins and chaperone protein complexes (page 15, lines 25-27). The specification elaborates that preferably, fractions containing grp94/gp90, hsp90, hsp70, and CRT are collected, and then pooled (page 15, lines 29-30).

The specification further states that where the isoelectric focusing (IEF) is conducted with a liquid matrix, fractions can be separately collected by applying an external force to a region within the matrix (page 15, lines 31-33). Then the specification states that the fractions can be pooled together (page 15, lines 34-35). Thus, in the methods of the invention, the step of collecting fractions may be followed by the step of pooling fractions.

The pending claims also distinguish between collecting fractions and pooling fractions. Claims 1, 10, and 11 refer to "collecting one or more fractions". Preparation of the sample of the pharmaceutical composition of claim 11 comprises a step that involves collecting fractions wherein the collected fractions "comprise a mixture of chaperone protein complexes;" such a collected fraction may contain a mixture of chaperone protein complexes not due to the mixing together of multiple fractions, but because chaperone protein complexes may aggregate and not separate completely (as described in the specification at page 30, lines 30-35), and so different chaperone protein complexes may be found in a single fraction. Claim 18 places a further limitation on the subject matter of claim 11, wherein the proteins from collected fractions are pooled. Thus, claim 18 distinguishes the steps of collecting fractions and pooling collected fractions.

Therefore, Applicants respectfully submit that the specification and claims clearly distinguish the separate steps of collecting fractions and pooling fractions, and as such, there is no confusion over the meaning of the recited phrases. The rejection of claims 1 and 10 under 35 U.S.C. § 112, second paragraph, thus should be withdrawn.

Claims 9, 10, and 17 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite in reciting trademarks of detergents, namely TRITON and IGEPAL. Applicants have amended these claims to specify the chemical name of the trademarked products of these claims. The chemical compositions of the trademarked detergents are inherent properties of the detergents and are not new matter (see Sigma catalog descriptions attached as Exhibit A).

Accordingly, Applicants respectfully request that the rejection of claims 9, 10, and 17 under 35 U.S.C. § 112, second paragraph, for recitation of the trademarks of detergents TRITON and IGEPAL, should be withdrawn.

3. THE CLAIM REJECTIONS UNDER 35 U.S.C. § 102(a) SHOULD BE WITHDRAWN

The Examiner rejects claims 1-20 under 35 U.S.C. § 102(a) as anticipated by Graner et al. (Cancer Immunol. Immunother. 2000, 49:476), hereinafter "Graner (Nov. 2000)". The Examiner contends that Graner (Nov. 2000) discloses the same process and product as instantly claimed.

In response, Applicant points out that the results in Graner (Nov. 2000) are Applicants' own work, and that Graner (Nov. 2000) was published in November of 2000, which is within one year of the effective filing date of the present application (i.e., May 1, 2001, on which date was filed U.S. Provisional Application Serial No. 60/287,967, from which the present application claims priority). In order for a reference to be considered prior art under 35 U.S.C. § 102 or § 103, it must have been published greater than one year prior to the filing date of the application against which it is applied. Applicants contend that Graner (Nov. 2000) is not available as prior art for any purpose under 35 U.S.C. § 102 or § 103. To support this contention, Applicants provide herewith a Declaration Under 37 C.F.R. § 1.132 By Dr. Michael Graner and Dr. Emmanuel Katsanis (the "Graner-Katsanis Declaration") as evidence that Graner (Nov. 2000) is the inventors' own invention, the publication of which occurred less than one year prior to the effective filing date of the present application.

In the Graner-Katsanis Declaration, Dr. Michael Graner and Dr. Emmanuel Katsanis state that they are the inventors of the claimed invention, and that the other co-authors of the Graner (Nov. 2000) publication, Amy Raymond and Emmanuel Akporiaye conducted experiments pursuant to their design, at their suggestion or under their supervision. *See* In re Katz, 687 F.2d 450, 215 U.S.P.Q. 14 (C.C.P.A. 1982).

In view of the facts set forth in the Graner-Katsanis Declaration, the Graner (Nov. 2000) reference is not the publication of another, and is not available as prior art for any purpose under 35 U.S.C. § 102 or § 103. Applicants therefore request that the rejection under 35 U.S.C. § 102(a) be withdrawn.

4. THE CLAIM REJECTIONS UNDER 35 U.S.C. § 102(b) SHOULD BE WITHDRAWN

The Examiner rejects claims 1, 3, 11, and 18-20 under 35 U.S.C. § 102(b) as anticipated by Katsanis et al. (Keystone Symposia on Cellular Immunity and Immunotherapy of Cancer, 2000, abstract 431), hereinafter "Katsanis". The Examiner contends that Katsanis discloses the same process and product as instantly claimed. The Examiner acknowledges that details of the procedure of the instant specification are not available in the abstract, but the Examiner contends that, as the pIs of the chaperones were known, FS-IEF would have inherently been performed using fractions in the claimed range. Applicants submit that Katsanis is not enabling for the methods of the claimed invention, and that inherent anticipation cannot be established.

An anticipatory reference must enable one skilled in the art to make the claimed subject matter at the time of the invention without undue experimentation. *See PPG Indus., Inc. v. Guardian Indus., Corp.*, 75 F.3d 1558, 1566, 37 U.S.P.Q. 2d 1618, 1624 (Fed. Cir. 1996). "A sufficient description is one that, in clear and exact terms, describes the invention with enough specificity to enable a person skilled in that field, at that time, prior to the critical date, 'to practice the invention.'" *Canron, Inc. v. Plasser Am. Corp.*, 474 F. Supp. 1010, 1013, 203 U.S.P.Q. 440, 444 (E.D. Va. 1978), *aff'd*, 609 F.2d 1075 (4th Cir. 1979), *cert. denied*, 446 U.S. 965 (1980).

Applicants submit that Katsanis teaches neither the claimed methods nor how to achieve the claimed products. Katsanis does not describe the invention with sufficient detail to enable the skilled artisan to practice the invention without undue experimentation.

The Examiner notes that Katsanis lacks "some of the details of the procedure". Applicants submit that Katsanis fails to teach any of the details of the procedure, and most significantly fails to teach the use of FS-IEF conditions involving chaotropic agents such as 6M urea (as well as the use of detergents such as Triton X-100, Triton X-114, and Igepal CA-630).

Indeed, armed with the knowledge in the art that the compounds of interest, desired for use as anti-cancer vaccines, were noncovalent complexes of chaperone proteins and peptides (see e.g. WO 97/10001 dated March 20, 1997; WO 96/10411 dated April 11, 1996), the skilled artisan would <u>not</u> have utilized chaotropic agents such as 6M urea during FS-IEF, because the presence of such chaotropic agents would have been expected to disrupt the noncovalent bond between the proteins and peptides, thereby destroying the immunogenicity of the vaccine. Applicants discovered the unexpected result that FS-IEF carried out in the presence of a chaotropic agent, instead of being contraindicated, is necessary to achieve successful FS-IEF of the chaperone-peptide complexes from tumor tissue. FS-IEF carried out in the presence of a chaotropic agent results in multimers or aggregates of chaperone protein-peptide complexes that co-focus with each other, and which did not migrate to the predicted or published isoelectric points of the chaperone proteins. The instant specification discloses that chaperone proteins isolated by the FS-IEF methods of the invention did not separate from each other, and instead formed multimers or aggregates (see the specification at page 31, lines 3-33).

Applicants invite the Examiner's attention to paragraphs 8.1-8.6 of the Graner Declaration. As described in the Graner Declaration, FS-IEF performed in the presence of a denaturing chaotropic agent such as 6M urea and detergents would be expected to inhibit hydrophobic interactions and reduce protein-protein interactions (*see* the Graner Declaration at ¶ 8.1). The expectation based on the knowledge in the art would have been that certain chaperones would separate into discrete fractions according to the pI of each chaperone and

that such chaperones would be further purified individually. The presence of high molar concentrations of urea would be expected to decrease protein binding and increase protein separation. Thus, the skilled artisan would not have expected that FS-IEF in the presence of a chaotropic agent such as urea would afford the successful enrichment of chaperone protein-peptide complexes. (*see* the Graner Declaration at ¶ 8.2). However, instead of migrating to the respective isoelectric points, it was observed that the chaperones formed high molecular-mass complexes that did not dissociate despite the presence of 6M urea. It was unexpected that the chaperones and associated antigenic peptides would coalesce together in the middle of the pH range in the presence of a chaotropic agent such as urea. It was also unexpected that they would coalesce in a relatively narrow range of pH (*see* the Graner Declaration at ¶ 8.3).

The invention as claimed relies on the surprising observation that FS-IEF performed on tumor tissue homogenates, using harsh denaturing conditions including a chaotropic agent, yields compositions enriched for complexes containing a range of chaperone protein-peptide complexes within a relatively narrow pH range. The FS-IEF technique under these conditions creates a composition containing multiple beneficial chaperones and peptides necessary to stimulate an effective immune response against the tumor, thereby providing an unexpectedly complete vaccine product in essentially one step (*see* the Graner Declaration at ¶ 8.4). Based on the knowledge in the art about FS-IEF performed using harsh denaturing conditions, it was unexpected that the chaperones did not separate but instead coalesced. The maintenance of chaperone protein-peptide complexes within the fractions that are enriched for chaperones was also unexpected, given the use of urea as a chaotropic dissociant (*see* the Graner Declaration at ¶ 8.5). As concluded in the Graner Declaration, without the teachings in the instant application, one of ordinary skill in the art would not (i) expect the above-described behavior of chaperone protein complexes in FS-IEF performed under harsh denaturing conditions, and (ii) appreciate that FS-IEF could be utilized under such conditions

to produce a composition in which multiple chaperones and associated peptides remain together in complex, thereby providing a vaccine product using a single-step enrichment method (*see* the Graner Declaration at ¶ 8.6).

Katsanis fails to teach the use of a chaotropic agent and fails to teach that the chaperone proteins migrated as aggregates or multimeric complexes rather than to the predicted or published pI's of the individual proteins. Therefore, Katsanis fails to teach one skilled in the art how to carry out the FS-IEF of chaperone-peptide complexes without undue experimentation. Katsanis thus fails to enable the claimed invention.

The Examiner states that this rejection is based in part on inherency, contending that FS-IEF would have inherently been performed using fractions in the known pI ranges of the individual chaperone proteins. As discussed above, the chaperone complexes did not focus to the known pI's of the individual chaperone proteins, and thus the Examiner's statement is erroneous.

In view of the foregoing, Katsanis does not anticipate the claimed invention, and the rejection under 35 U.S.C. § 102(b) should be withdrawn.

5. THE CLAIM REJECTIONS UNDER 35 U.S.C. § 103(a) SHOULD BE WITHDRAWN

The Examiner rejects all claims under 35 U.S.C. § 103(a) as obvious over Katsanis et al. (Keystone Symposia on Cellular Immunity and Immunotherapy of Cancer, 2000, abstract 431, "Katsanis") and Graner et al. (Cancer Immunol. Immunother. 2000, 49:476, "Graner (Nov. 2000)") in view of Graner et al. (Clin. Cancer Res. 2000, 6:909), hereinafter "Graner (Mar. 2000)", Lucietto et al. (J. Peptide Res. 1997, 49:308), hereinafter "Lucietto", and Rotofor™ System Bio-Rad Tech Notes Summaries, hereinafter "Bio-Rad". According to the Examiner, Graner (Mar. 2000) combines purified chaperones into a mixture, Lucietto discloses purification of chaperonin-10 using IEF and Bio-Rad discloses detergents and denaturants to purify proteins. The Examiner alleges that the skilled artisan would be

motivated to use detergents and denaturants in IEF to purify chaperones. Applicants respectfully disagree.

A finding of obviousness under 35 U.S.C. § 103 requires a determination of the scope and the content of the prior art, the differences between the invention and the prior art, the level of the ordinary skill in the art, and whether the differences are such that the claimed subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. Graham v. Deere, 383 U.S. 1 (1966). The relevant inquiry is whether the prior art suggests the invention, and whether one of ordinary skill in the art would have had a reasonable expectation that the claimed invention would be successful. In re O'Farrell, 853 F.2d 894, 902-4 (Fed. Cir. 1988); In re Vaeck, 947 F.2d 488, 20 U.S.P.Q. 2d 1438 (Fed. Cir. 1991). Both the suggestion of the claimed invention and the expectation of success must be in the prior art, not in the disclosure of the claimed invention. In re Dow Chemical Co., 5 U.S.P.Q. 2d 1529 (Fed. Cir. 1988).

Applicants respectfully point out that Graner (Nov. 2000) is not applicable as prior art under 35 U.S.C. § 102 and § 103 as discussed *supra*, and thus the references addressed below for this obviousness rejection are Katsanis, Graner (Mar. 2000), Lucietto, and Bio-Rad.

Applicants contend that the claims are not rendered obvious by the combination of Katsanis, Graner (Mar. 2000), Lucietto, and Bio-Rad for the reasons discussed below.

The Examiner admits that Katsanis lacks all details of the procedure. In particular, Katsanis does not teach the unexpected aspects of the invention, that FS-IEF is carried out in the presence of a chaotropic agent, and that the chaperone protein-peptide complexes migrate as aggregates or multimers rather than focusing to the pI's of individual chaperone proteins. Thus there is no suggestion of the claimed methods in Katsanis and no reasonable expectation of success in achieving the claimed methods or products. None of the other references cited by the Examiner remedies the deficiencies in Katsanis's disclosure, even could they be properly combined with Katsanis.

Graner (Mar. 2000) teaches separation and purification of chaperone proteins, isolated individually, by a multi-step method other than FS-IEF, followed by using the different chaperone types separately to immunize mice challenged with A20 B-cell leukemia. Applicants submit that Graner (Mar. 2000) does not make obvious the product or process of the claimed invention. Graner (Mar. 2000) does not teach or even suggest to use FS-IEF to produce chaperone protein-peptide multimeric complexes. Graner (Mar. 2000) does not teach or suggest isolation or use of chaperone protein aggregates, or that FS-IEF would produce such complexes. Graner (Mar. 2000) does not teach FS-IEF conditions using chaotropic dissociants such as 6M urea to produce FS-IEF fractions enriched for the chaperone protein complexes of the invention. Therefore, Applicants submit that Graner (Mar. 2000) does not remedy the deficiencies in teachings of Katsanis, since it also provides no suggestion or reasonable expectation of success in achieving the claimed methods and products.

Lucietto teaches the use of IEF to purify a synthetic polypeptide after chemical synthesis. Applicants invite the Examiner's attention to paragraphs 9.1-9.2 of the Graner Declaration. Lucietto utilizes IEF as a means of purifying a single synthetic protein or protein fragment of interest from contaminating by-products of chemical synthesis (*see* the Graner Declaration at ¶ 9.1). Lucietto starts with nearly purified product (the target of synthesis) and uses two rounds of IEF to purify the synthetic protein/fragment to homogeneity. Lucietto teaches that the buffer condition used in their method prevents aggregation of proteins (*see* the Graner Declaration at ¶ 9.2), as stated in Lucietto on page 318, column 2, numbered paragraph 4: "The IEF technique is compatible with the use of non-ionic denaturing agents (e.g., NONIDET, urea) which on the one hand allow the loading in the ROTOFOR chamber (50 mL) of relatively large quantities (30-100 mg) of even poorly soluble proteins and on the other avoid precipitation of the latter near or at its pIs and the formation of aggregated species which may precipitate or focus at a different pH." (emphasis

added). There is no suggestion or motivation in Lucietto to use FS-IEF for enriching a mixture of different chaperone-peptide complexes from a biological sample.

Moreover, Lucietto is improperly combined with Katsanis, Graner (Mar. 2000), and Bio-Rad, because the procedures in Lucietto involve isolation of a single polypeptide, rather than a multimeric protein complex. As described in the Graner Declaration, one of ordinary skill in the art would not have applied the teachings of Lucietto or Bio-Rad, directed to purifying individual, noncomplexed polypeptides, to a method to produce the enriched chaperone-peptide complexes of the invention, because the presence of a chaotropic agent such as urea would be expected to reduce or abolish protein-protein interactions (*see* the Graner Declaration at ¶ 9.4). Using the methods of the claimed invention, chaperone proteins do not separate from each other and from their complexed peptides but instead form high molecular weight complexes. The maintenance of antigenic peptides within the fractions that are enriched for chaperones would not be expected, given the use of urea as a denaturant/chaotropic dissociant and the presence of detergents (*see* the Graner Declaration at ¶ 9.4). Therefore, one of skill in the art would not have combined the teachings of Katsanis, Graner (Mar. 2000), and Bio-Rad, to produce the chaperone-peptide complexes of the invention. The methods and the motivation behind using FS-IEF as disclosed in Lucietto, and the purified protein product resulting therefrom, differ substantially from the methods of the instant specification for enriching multiple chaperone-peptide complexes. Lucietto does not provide a suggestion of the claimed method. Nor does Lucietto provide a reasonable expectation of success of the claimed invention even when combined with Katsanis and/or the other references. Therefore, Lucietto does not make obvious the product or process of the claimed invention.

Bio-Rad discloses the use of detergents and urea in FS-IEF for the purification of certain proteins. However, Bio-Rad does not teach the use of a chaotropic agent in FS-IEF for isolating noncovalent protein complexes.

Applicants submit that, as outlined in the Graner Declaration at ¶ 9.5, the skilled artisan following the teachings of Katsanis, Graner (Mar. 2000), Lucietto, and Bio-Rad, and the knowledge in the art, would not have been motivated to use the methods of the claimed invention because none of these references teaches the use of FS-IEF in the presence of a chaotropic agent to enrich for noncovalent complexes of chaperone and peptide, which association of chaperone and peptide was known to be necessary for immunogenicity. The conditions that allowed the FS-IEF procedure to yield a viable product, namely the use of urea and detergents, would be expected to result in the loss of complex formation and thus loss of antigenic peptides that provide the specificity necessary to mount an adaptive immune response against a tumor (*see* the Graner Declaration at ¶ 9.5).

In view of the foregoing, Applicants assert that the combination of Katsanis, Graner (Mar. 2000), Lucietto, and Bio-Rad does not render obvious the methods or products of the claimed invention. As such, Applicants request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

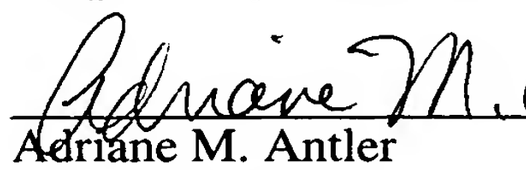
CONCLUSION

Applicants believe that the present claims meet all the requirements for patentability. Entry of the foregoing amendments and remarks into the file of the above-identified application is respectfully requested. Withdrawal of all rejections and reconsideration of the amended claims are requested. An allowance is earnestly sought.

If any issues remain, the Examiner is requested to telephone the undersigned.

Respectfully submitted,

Date: July 8, 2004



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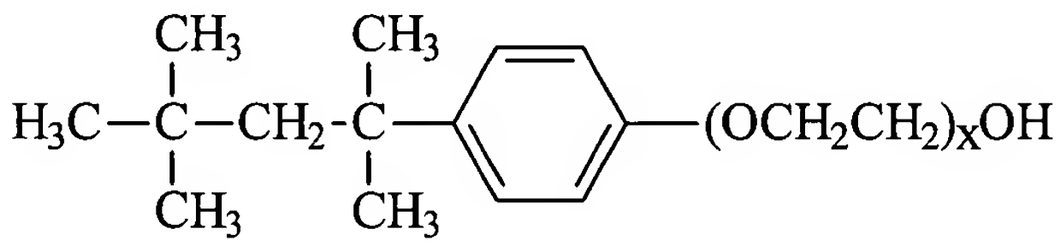
ProductInformation

TRITON X-100
Product Number **X-100, T9284, T8787, T8532**
Storage Temperature 25°C

CAS #: 9002-93-1
Synonyms: X-100; TRITON X-100[1]; Octylphenol ethylene oxide condensate[1]

Product Description



x = 9-10

Appearance: Liquid, clear to slightly hazy, colorless to light yellow
Specific gravity: 1.065 at 25°C (approx. 1.07 g/mL)[1]
Approximate Molecular Weight: 625[1];
effective molarity =1.7 M for the neat liquid.[1]
UV absorption: λ_{max}= 275 nm ($E^{1\%}$ = 23.9) and 283 nm ($E^{1\%}$ = 19.4) in methanol[2]

Typical values:
Viscosity (Brookfield): 240 cps at 25°C[1]
Cloud point (1% aqueous solution): 63-69°C[1]
Pour point: 7°C[1]
pH (5% aqueous solution): 6.0 to 8.0[1]
Calculated HLB value: 13.5[1]
Critical micelle concentration (CMC): 0.22 to 0.24 mM[3,4]

The structure of Triton X-100 is very similar to that of Igepal CA-630 (Sigma I3021) and of Nonidet P-40 (no longer commercially available); the names are sometimes reported as synonyms.[5] However, Triton X-100 is slightly more hydrophilic than Igepal CA-630. The two are not considered to be functionally interchangeable in most applications.

Triton X-100 is a nonionic detergent, 100% active ingredient, which is often used in biochemical applications to solubilize proteins. Triton X-100 has no antimicrobial properties.[1] It is considered a comparatively mild non-denaturing detergent and is reported in numerous references. It does absorb in the ultraviolet region of the spectrum, so it can interfere with protein quantitation by absorption at A_{280nm}. A number of polymeric resins have been used to remove X-100 from solution, including Amberlite hydrophobic XAD resins[6] and Rezorian A161 cartridges.[3]

The "Triton X" series of detergents are produced from octylphenol polymerized with ethylene oxide. The number ("-100") relates only indirectly to the number of ethylene oxide units in the structure. X-100 has an "average of 9.5" ethylene oxide units per molecule, with an average molecular weight of 625.[1,3] In addition, lower and higher mole adducts will be present in lesser amounts, varying slightly within supplier's standard manufacturing conditions. A by-product formed during the reaction is polyethylene glycol, a homopolymer of ethylene oxide. Acid is also added to the product to neutralize the product after the base catalyzed reaction is completed. No antioxidants are added by Sigma or the manufacturer, but commercial preparations of Triton X-100 have been found to contain peroxides up to 0.22% hydrogen peroxide (H_2O_2) equivalents,[7] which may interfere with biological reactions. Sigma offers X-100-PC and X-100R-PC as alternatives. Triton X-100 absorbs in the UV spectrum at approximately the same wavelength as proteins (280 nm). Sigma offers two "reduced", i.e., hydrogenated forms, X-100-RS and X-100R-PC that have significantly lower absorbance in this region.[4]

For lysing cells, typically about 0.1% X-100 solution in water will be sufficient, and even up to 0.5% concentrations will usually not harm most enzymes being isolated.[3] Many enzymes remain active in the presence of X-100; for example, Proteinase K, remains active in 1% (w/w) solutions of X-100.[8]

Triton X-100 can be detected in the parts per million range by spectrophotometric measurement of the concentration of a Triton-ammonium-cobalt-thiocyanate complex.[9] Interfering substances in this assay have been discussed.[15]

For a given application, the choice of a suitable surfactant depends on a number of variables, from its solubility, polarity and micelle size to the mechanism of its action with the target solute. The literature contains numerous articles:[10-13]

- Choice of detergent for solubilization of (erythrocyte) membranes[10]
- Effect of hydrophile-lipophile balance on (cytochrome) membrane solubilization[11]
- Mode of interaction of polyoxyethylene glycol detergents with membrane protein[12]
- General background on surfactants and use in protein purification[13]

Preparation Instructions

Triton X-100 is soluble in all proportions at 25°C in water, benzene, toluene, xylene, trichloroethylene, ethylene glycol, ethyl ether, ethanol, isopropanol, and ethylene dichloride.[1] At 10% (v/v) in water, it gives a clear to slightly hazy solution, from clear to slightly yellow in appearance.[3]

Solutions are stable to autoclaving. At certain concentrations the solutions may be cloudy but dispersible above the cloud point; they should clear with stirring upon cooling.[14]

Storage/Stability

Triton X-100 is considered stable for years if stored sealed at room temperature. For special applications, storage under argon or nitrogen at 2-8°C may be preferred.

References

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9. Supplier method, based on work by Crabb, N.T. and Persinger, H.E., J. Amer. Oil Chem. Soc., 41, 752-755 (1964) and Greff, R.A. et al., J. Amer. Oil Chem. Soc., 42, 180-185 (1965).
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13. Methods in Enzymology, 182, 239-282 (1990): reviews by Neugebauer, J.M., Hjelmeland, L.M., etc.
14. Supplier information.
15. Goldstein, S. & Blecher, M., Anal. Biochem., 64, 130-135, (1975).

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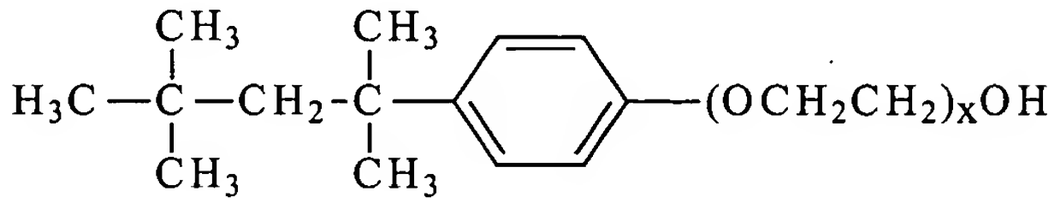
ProductInformation

TRITON X-114
Product Number **X-114**
Storage Temperature RT°C

CAS #: 9036-19-5
Synonyms: tert-octylphenoxypoly (ethoxyethanol); polyoxyethylene monooctylphenyl ether; octylphenoxypoly (ethyleneoxy) ethanol; tert-octylphenoxy poly(oxyethylene)ethanol

Product Description



x = 7-8

Appearance: Clear, pale-straw-colored liquid
Average Molecular weight: 537 (based on 7.5 ethylene oxide units)
Specific gravity: 1.05-1.06 at 25°C[1]
Brookfield viscosity (at 25°C): approximately 260 cps[1]
Cloud point: 22-28°C; for 1% solution in water, 20-22°C[1]
CMC value: Approximately 0.2 mM or 0.009% (w/w in water)
HLB value (calculated): 12.4[2]
Pour point: -9°C (16°F)
pH of 5% aqueous solution: 6.0-8.0[1]
Absorbance: 277 nm, $E^{1\%}$ = 26-29 (more commonly 27.5-28.7)[3]

Triton X-114 is a nonionic detergent, 100% active ingredient, that is often used in biochemical applications to solubilize proteins. The "X" series of Triton detergents are produced from octylphenol polymerized with ethylene oxide.

At temperatures above the cloud point of a surfactant, solutions separate into aqueous and detergent-enriched phases. It is this property that makes Triton X-114 particularly useful in separating lipophilic proteins from hydrophilic proteins.[4,5] For example, HMG-CoA reductase was recovered quantitatively in an aqueous phase of a biphasic system formed by Triton X-114 at 30°C.[6] The cloud point of a 1% (w/w) X-114 aqueous solution is increased substantially (to about 50°C) in the presence of small amounts of ionic surfactants (1.25 mM SDS or CTAB). Adding electrolytes to a Triton X-114 solution does decrease the cloud point, the effect being nearly a linear function of the concentration. The combination of electrolyte and ionic surfactant causes a drastic reduction in the cloud point of the Triton X-114 solution.[7]

Preparation Instructions

Triton X-114 is soluble at 1 g/10 mL in ethanol,[2] but does have some solubility in cold water at least to 5% (w/w). (Above the cloud point, phase separation occurs.) It is insoluble in aliphatic hydrocarbons, but miscible in all proportions in aromatic hydrocarbons and polar organic solvents. It is soluble in hydrochloric, phosphoric and dilute sulfuric acid, but not in concentrated sulfuric acid solutions.[1]

Storage/Stability

Kept sealed, the product is stable at room temperature for years. Any substance containing ether linkages, however, is subject to the formation of peroxides. For sensitive applications, storage under inert gas at 2-8°C is recommended.

References

1. Supplier data. Triton is a trademark name owned by Union Carbide Company.
2. Helenius, A. and Simons, K., Biochim. Biophys. Acta, 415, 29-79 (1975).
3. Sigma quality control.
4. Bordier, C., J. Biol. Chem., 256, 1604-1607 (1981).
5. Neugebauer, J., Meth. Enzymol, 182, 247-249 (1990).
6. Concepcion, J.L. et al., Arch. Biochem. Biophys., 352, 114-120 (1998).
7. Gu, T. and Galera-Gomez, P.A., Colloids and Surfaces A: Physicochem. Eng. Aspects, 104, 307-312 (1995).

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ProductInformation

IGEPAL
Product Number **I3021**

CAS #: 9002-93-1
Synonyms: tert-Octylphenoxy poly(oxyethylene)ethanol

Product Description
Appearance: Clear to slightly hazy, viscous liquid
Brookfield viscosity (25°C): Approx. 300 cps
pH (5% aqueous solution): 6.0-8.0
Cloud point: 63.0 to 67°C (1% solution)
Pour point: Approx. 7°C
Neutralization number: Maximum 0.5 mg KOH/g (at pH 7)
Specific gravity: 1.06
HLB (hydrophilic/lipophilic balance): 13.0
CMC (Critical micelle concentration): 0.083 mM (or 0.005% v/v)
Average EO (number ethylene oxide units/molecules: 9
Approx. Molecular Weight: 603

Igepal is a trademarked surfactant manufactured by Rhodia (as of January 1998, a separate company owned by Rhone-Poulenc). Igepal CA-630 was offered by Sigma-Aldrich in 1995 as a replacement for Nonidet P-40 (formerly Sigma Prod. No. N6507) when Shell ceased manufacture. Since 1995, customer acceptance of Igepal CA-630 in a wide range of biochemical applications has been excellent. Usage of I3021 in lysing buffers, etc., has been identical to that for Nonidet P-40.

Please note that Igepal, Nonidet P-40, and Triton X-100 (all commonly cited in biochemical literature) are liquid surfactants of similar structure and average formula weight: octylphenols with slightly different ethylene oxide values (9 to10). References to Nonidet P-40 as "NP-40" have occasionally led to the use of a different product Tergitol NP-40, a nonylphenol surfactant (with approximately 40 EO units) that is a solid at room temperature.

Special application-tested Igepal CA-630 products are available as well:
Molecular Biology tested: I8896 has replaced N0896.
Electrophoresis grade: I7771 has replaced N3268.

Preparation Instructions
This product is miscible in all proportions with water. It may be helpful to prepare a dilute stock solution (10-25%), to be diluted further at time of use. Solutions stored at 2-8°C are stable at least a month, but it is important to minimize exposure to oxygen. If formation of trace peroxides is critical to usage, storage under inert gas at 2-8°C is advised. Preservatives may be added to the solution to prevent bacterial growth.

Storage/Stability
Igepal CA-630 is stable for at least three years stored at room temperature. Over time the material may develop a sediment, particularly if stored below 20°C. The sediment is merely the high-end molecular weight fraction beginning to solidify. If the material is warmed to 40-50°C and mixed gently, the Igepal will again be a clear liquid.[1,2]

Any detergent with ethylene oxide units will develop trace peroxides over time if exposed to air. For sensitive oxidation-reduction applications, users may wish to store opened containers under inert gas at 2-8°C.

References
1. Supplier data.
2. Sigma data.
3. Merck Index, 12th ed. (1996).

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